The species concept in *Aspergillus:* recommendations of an international workshop

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Overview

Introduction

What is a species

Outcome of the International Aspergillus workshop

Aspergillus naming

Conclusion





What is a species in *Aspergillus*?

Morphological characters Genotype Ecology Physiology Metabolites Clinical significance Etc.

Aspergillus systematics in the genomic era An international workshop



12 - 14 April 2007 CBS Fungal Biodiversity Centre Utrecht – The Netherlands

Theme of the Workshop What is a species and how do we define the species in Aspergillus ? Fundamental versus applied Species concept -Phylogeny and identification Aspergillus nomenclature

Topics which were discussed

- What is the impact of *Aspergillus* taxonomy in terms of epidemiology, case definitions and biological understanding of disease?
- What and how many genes should be used to delimit an *Aspergillus* taxon?
- How does the phylogenetic species concept translate to practical and routine diagnoses?
- What are the roles of *Aspergillus* databases for species identification?
- What is the value and impact of polyphasic approaches for species identification?
- What genes/methods can be used to design kits for rapid identification?
- How should new species be proposed?

Currently used species concepts

Delimitation of species based only on morphology is difficult due to variation of colony pattern and conidial structures

Physiological characters

Extrolite characters

Molecular characters

Polyphasic taxonomy seems to be the gold standard





Species concept in section *Fumigati* Based on a polyphasic approach Using morphology Extrolite profiles and Phylogenetic characters (β-tubulin, calmodin, actin and ITS)

The section now comprises 33 taxa: 10 strictly anamorphic and 23 Neosartorya teleomorphs

Samson et al. (2007). Studies in Mycology 59



| Table 2. Extrolites | produced by species assigned to Aspergillus section Fumigati |
|-----------------------------|---|
| Species | Extrolites produced |
| Aspergillus brevipes | roquefortine C, cf. meleagrin |
| Aspergillus duricaulis | pseurotin A, fumagillin, asperpentyn, duricaulic acid and asperdurin, |
| | phthalides, chromanols, cyclopaldic acid, 3-O-methylcyclopolic acid |
| Aspergillus fumigatiaffinis | auranthine, cycloechinuline, fumigaclavines, helvolic acid, neosartorin, |
| | palitantin, pyripyropenes A, E, O & S, tryptoquivalins, tryptoquivalons |
| Aspergillus fumigatus | fumagillin, fumitoxins, fumigaclavines A &C, fumitremorgins, gliotoxin, |
| | trypacidin, pseurotins, helvolic acid, pyripyropens, methyl-sulochrin, |
| | verruculogen, fumiquinazolines |
| Aspergillus fumisynnematus | neosartorin, pyripyropens, fumimycin |
| Aspergillus lentulus | cyclopiazonic acid, pyripyropenes A, E & O, terrein, auranthine, |
| | neosartorin |
| Aspergillus novofumigatus | aszonalenin, cycloechinuline, fiscalins, helvolic acid, neosartorin, |
| | palitantin, terrein, territrem B |
| Aspergillus turcosus | kotanins and several unique but not yet elucidated secondary |
| | metabolites |
| Aspergillus unilateralis | mycophenolic acid, other unique secondary metabolites |
| Aspergillus viridinutans | viriditoxin, 13-O-methylviriditin, phomaligin A, variotin, viriditin, |
| | wasabidienone B0, B1, viriditin, 4-acetyl-6,8-dihydroxy-5-methyl-2- |
| | benzopyran-1-1 A |
| Neosartorya assulata | indole alkaloids and apolar metabolites |
| Neosartorya aurata | helvolic acid, yellow unidentified compounds |
| Neosartorya aureola | fumagillin, tryptoquivalins, tryptoquivalons, pseurotin A and viriditoxin |
| | (FRR 2269 also produces helvolic acid) |
| Neosartorya australensis | cf. wortmannin, cf. aszonalenin |
| Neosartorya coreana | aszonalenins |
| Neosartorya denticulata | gliotoxin, viriditoxin |
| Neosartorya fennelliae | asperfuran, aszonalenin, fumigaclavine, viridicatumtoxin |
| Neosartorya ferenczii | asperfuran, aszonalenin, fumigaclavine, viridicatumtoxin, cf. gliotoxin, |
| | fumigatins, cf. aszonalenin |
| Neosartorya fischeri | terrein, fumitremorgins A & C, tryptoquivaline A, trypacidin, TR-2, |
| | verruculogen, sarcin, aszonalenins, fischerin, neosartorin, fiscalins, |
| | helvolic acid |
| Neosartorya galapagensis | gregatins |
| Neosartorya glabra | asperpentyn, avenaciolide, wortmannin-like compound |
| Neosartorya hiratsukae | avenaciolide |
| Neosartorya laciniosa | aszonalenins, tryptoquivalins, tryptoquivalons |
| Neosartorya multiplicata | helvolic acid |
| Neosartorya papuensis | cf. wortmannin |
| Neosartorya pseudofischeri | asperturan, cytochalasin-like compound, fiscalin-like compound, |
| | pyripyropens, gliotoxin |
| Neosanonya quadricincta | quindiactacin, aszonalenins |
| Neosartorya spinosa | aszonalenins, 2-pyrovoylaminobenzamide, pseurotin |
| Neosartorya spathulata | xanthocillins, aszonalenins |
| Neosartorya stramenia | quindactacin, avenacidide |
| Neosartorya tatenoi | aszonalenins |
| Neosartorya udagawae | Fumigatin, tumagillin, tryptoquivalins, tryptoquivalons |
| Neosartorya warcupii | ct wortmannin, ct. aszonalenin, cf. chromanols, cf. tryptoquivalins and |
| | cf. tryptoquivalons |

Extrolites of section Fumigati

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Table 2. Extrolites produced by species assigned to Aspergillus section Fumigati

Extrolites of section Fumigati

Polyphasic taxonomy other examples





Section Clavati

Section Usti





Section Nigri with 19 accepted taxa

Samson et al. (2007). Studies in Mycology 59

RECOMMENDATIONS

- A polyphasic approach is recommended for describing new *Aspergillus* species, including molecular, morphological, physiological and ecological data.
- Any proposed new species should show evidence for evolutionary divergence from other taxa, particularly unique DNA characters at multiple loci, in addition to any distinctive extrolites and morphological characters.
- Any new species should be compared to type strains of presumed relatives.
- For the description of new taxa, multiple, independent loci are recommended for use, particularly loci for which large datasets already exist, such as ITS, β-tubulin, calmodulin, actin, RNA polymerase 2.
- Detailed morphological and physiological descriptions should be provided.
- Media used for the description should be based on the use of media recommended by the International Commission of *Penicillium* and *Aspergillus*: Malt Extract Agar and Czapek's Agar, with referenced formulas.

RECOMMENDATIONS

- Type cultures of new *Aspergillus* species should be deposited in at least two international recognized culture collections.
- If type cultures are not made available for the scientific community, the species will be considered invalid.
- New species names should be registered at MycoBank (<u>www.MycoBank.org</u>).
- Sequences must be deposited in recognized genetic databases.
- Use dual names where necessary, and a single name in normal use, depending on the state (teleomorph or anamorph) that is observed.
- Focused databases that are linked to other related databases are encouraged, with links giving an idea about the content of the database.
- A simple database for identification was proposed which would include basic sequences, photos, links to media/growth protocols, or the possibility to make a phylogenetic tree.

This protocol will be endorsed by the IUMS International Commission of *Penicillium* and *Aspergillus*.



Aspergillus names

- Names in *Aspergillus* have an important impact in Applied Research and Biotechnology
- Important is to stabilize names and avoid confusion in the applied fields

Rules

- Correct name
- Latin description
- Designation of holotype
- Publication



Description of new taxa

Current rules are described in the International Code of Botanical Nomenclature

> INTERNATIONAL CODE OF BOTANICAL NOMENCLATURE (VIENNA CODE)

> > 2006

SECTION 2. CONDITIONS AND DATES OF VALID PUBLICATION OF NAMES

Article 32

32.1. In order to be validly published, a name of a taxon (autonyms excepted) must: (a) be effectively published (see Art. 29-31) on or after the starting-point date of the respective group (Art. 13.1); (b) be composed only of letters of the Latin alphabet, except as provided in Art. 23.3 and Art. 60.4, 60.6, 60.9, and 60.10; (c) have a form which complies with the provisions of Art. 16-27 (but see Art. 21.4 and 24.4), and Art. H.6 and H.7; (d) be accompanied by a description or diagnosis or by a reference to a previously and effectively published description or diagnosis (except as provided in Art. 42.3, 44.1, and H.9); and (e) comply with the special provisions of Art. 33-45 (see also Art. 61).

32.2. A diagnosis of a taxon is a statement of that which in the opinion of its author distinguishes the taxon from other taxa.

Ex. 1. "Egeria" (Néraud in Gaudichaud, Voy. Uranie, Bot.: 25, 28, 1826), published without a description or a diagnosis or a reference to a former one, was not validly published.

Ex. 2. "Loranthus macrosolen Steud." originally appeared without a description or diagnosis on the printed labels issued about the year 1843 with Sect. II, No. 529, 1288, of Schimper's herbarium specimens of Abyssinian plants; the name was not validly published, however, until Richard (Tent. FL Abyss. 1: 340, 1847) supplied a description.

**Ex. 3* In Don, *Sweet's Hortus britannicus*, ed. 3 (1839), for each listed species the flower colour, the duration of the plant, and a translation into English of the specific epithet are given in tabular form. In many genera the flower colour and duration may be identical for all species and clearly their mention is not intended as a validating description or diagnosis.



Dual Naming

- There is a trend to have a one name-one fungus nomenclature
- For applied research this is difficult to accept



Aspergillus fumigatus Do we have to call this fungus *Neosartorya fumigata*?

Examples of two teleomorphs with two names



Neosartorya (Aspergillus) laciniosa



Neosartorya (Aspergillus) udagawae

Proposal for describing new taxa in Aspergillus and their teleomorphs

- Type cultures of new Aspergillus and teleomorph species should be deposited in at least two international recognized culture collections
- Type cultures should be available directly after the description has been published. If type cultures are not available for the scientific community within six months after the description the species will be considered invalid
- Latin descriptions can be short indicating differences with related taxa
- Good morphological and physiological descriptions are essential
- The new species name should be registered at MycoBank (see www.Mycobank.org)

RECOMMENDATIONS

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- If type cultures are not made available for the scientific community, the species will be considered invalid.
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- Sequences must be deposited in recognized genetic databases.
- Use dual names where necessary, and a single name in normal use, depending on the state (teleomorph or anamorph) that is observed.
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www.studiesinmycology.org

www.cbs.knaw.nl



Conclusion

Recommendations for an *Aspergillus* species concept proposed by an international panel is unique in mycology and is important for further research, not only in clinical microbiology but also in other fields (food, indoor environments, biotechnology etc.)

A polyphasic taxonomic approach and multilocus sequencing will clarify species and give a better understanding of the biodiversity in *Aspergillus*

The Aspergillus community should work together to achieve a stable and reliable taxonomy and make data available by good databases.